Passage of molecules through the wall of the gastrointestinal tract

II. Application of low-molecular weight polyethyleneglycol and a deterministic mathematical model for determining intestinal permeability in man

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SUMMARY The intestinal permeability to low molecular weight polyethyleneglycol (PEG) has been evaluated by means of a simple mathematical model and computer-aided curve-fitting procedures. Macrogol 400, a mixture of 11 PEGs with molecular weights ranging from 194 to 634 daltons, was taken together with a liquid meal and a six-hour portion of urine collected. The different PEGs were then extracted from the urine, separated from each other by gas-liquid chromatography, and the relative peak area of each individual PEG determined. The distribution of different PEGs in the urine was then compared with the original PEG-distribution in three different ways: (1) by comparing the median values of the molecular weights, (2) by comparing the mean and standard deviation after curve fitting to the normal distribution, and (3) by curve fitting to mathematical filter functions demonstrating molecular exclusion due to size. It thus appeared that molecules were excluded both in the high and in the low molecular weight range, possibly by a combined effect of the intestinal permeability barrier and an escape to other compartments than the urine. However, relatively more of the larger PEGs passed from the intestine to the urine in a patient with Crohn’s disease than in an apparently healthy individual.

Mucosal integrity is of prime importance in maintaining a healthy state in man. Accordingly, the mucosal membranes of the mature gut are relatively impermeable to large molecules. The mucosal coat, the unstirred-water layer, and the enterocyte plasma membrane, all form a barrier to molecular passage. Thus, the unstirred-water layer is rate-limiting for the passage of small, hydrophobic substances, whereas the enterocyte membrane forms a permeability barrier for more hydrophilic compounds. However, there is now increasing evidence that even large molecules may be transmitted across the wall of the mature gut. Such molecules—that is, proteins and immune complexes—are taken up into the intestinal epithelial cells by pinocytosis, and even larger compounds may be transmitted by way of 'persorption'.

It is, thus clear that a variety of molecules can traverse the mature mammalian gut during physiological conditions. Nevertheless, altered intestinal absorption may be of critical importance in the pathogenesis and pathophysiology of various disease states—both local inflammatory processes in the intestinal wall and general immunopathological conditions that may develop in due course. By implication, altered intestinal permeability for food constituents and microbial components may underlie the pathogenesis of a variety of diseases, both in the intestine and at distant sites such as the liver and joints.

A variety of compounds have therefore been used to delineate the permeability characteristics of mucosal membranes in health and disease. These include urea, erythritol, mannitol, and lactulose, inulin, and creatinine. Recently, Chadwick and his colleagues introduced a mixture of low molecular weight polyethyleneglycols as 'ideal' probe molecules for measuring intestinal permeability: polyethyleneglycol (PEG) is non-toxic, not degraded by intestinal bacteria, not metabolised during or after passage through the intestinal wall,
and rapidly excreted in the urine. The different-sized molecular components cross the intestinal epithelium at different rates, allowing characterisation of the passive permeability properties of the mcosa.

We have adopted the method proposed by Chadwick and his colleagues for studying normal and abnormal intestinal permeability states in man. At first, we reduced the complexity of the experimental situation by capitalising on a given anticipation\textsuperscript{22}.\textsuperscript{23}. Thus, although the absolute absorption of any single molecular weight species in the PEG mixture is dependent on such diverse factors as gastric emptying time, intestinal transit, intestinal permeability, and renal excretion, the relative absorption of the different molecules is dependent on intestinal permeability alone. If this were true, and provided the urinary excretion gives a true measure of the relative absorption, a single comparison of the ratio of different-sized molecules in the urine could be used as a reliable index of permeability. However, when these considerations were applied to the study of intestinal permeability in health and disease, we were consistently faced with the finding that there were more PEG molecules of intermediate than of small size in the urine. We have therefore invented a deterministic mathematical model to elucidate the characteristics of both intestinal and, by implication, non-intestinal filters through which PEG must pass between the gut and the urine. The present communication describes this mathematical model and its application in a healthy state and in a patient with Crohn's disease. On the basis of data presented, we propose that the patient with Crohn's disease had an impaired intestinal barrier.

Methods

CHEMICALS AND EQUIPMENT

Polyethyleneglycol with an average molecular weight of about 400 (PEG 400, HO-(CH$_2$-CH$_2$O)$_n$H, n=4-14) was obtained as Macrogolum 400 from Apoteksbolaget, Sweden. MB-3 mixed ion-exchange resin was obtained from Malinkrodt, St. Louis, Missouri, USA, and column-packing material—OV1 on Gas-Chrom O (30 g/l)—from Ohio Valley Spec. Chem. Inc., Marietta, Ohio 45750, USA. Other materials used were silanised glass-wool (Varian, No. 82-006000-00), acetic anhydride (Merck, No. 42), dimethyldichlorosilane (Merck, No. 803452) and toluene (Merck, No. 8325). The gas chromatograph was a Varian Aerograph series 1400, equipped with a flame-ionisation detector. Columns were made of glass 1.8 m (6 ft) long with an external diameter of 3.2 mm (1/8 in.) and an internal of 2 mm (Varian, No. 11-000081-03).

PROCEDURES

Subjects

The aim of the present study was to investigate the possible use of PEG 400 to get a deterministic, mathematical filter function that would describe the intestinal barrier in health and disease. For this reason, interest was initially focused on (1) a healthy individual (male, 30 years of age) with no signs or symptoms of renal or intestinal disease and (2) a patient with Crohn's disease (male, 22 years of age). Clinical and laboratory data revealed that the patient had suffered from classical Crohn's disease since 1974—that is, at least three years. An ileocaecal resection and an ileotransversostomy had been done in 1976. After symptom relapse in 1977, the distal ileum and part of the colon transversum were resected. However, symptoms still recurred in late 1977 and a 50 cm portion of the small intestine was severely affected by the disease. The current data about his condition were: daily abdominal pains, 10-15 diarrhoeal motions per day, SR 19 mm, Hb 140 g/l, and WBC 8.5 x 10$^6$/l. The subjects were given 10 g PEG 400 together with a liquid meal. A six-hour portion of urine was collected in clean, dry polyethylene plastic containers. The urine was then analysed for PEG as described below.

Extraction of PEG from urine

Ten millilitres of the six-hour-urine was put into a 50 ml-Pyrex test tube containing 12 ml MB-3 resin. The contents of the tube were mixed with a Vortexer, allowed to stand for five minutes at room temperature, revortexed, and again left for five minutes. The mixture was then filtered through paper (Munktell No. 5), Stora Kopparberg, Sweden) and freeze-dried overnight. The dry powder was dissolved in 2 ml acetic anhydride by vigorous shaking and left for 45 minutes at room temperature before transfer to 2 ml screw-cap ampoules. To obtain a reference at each extraction, 0.5 g PEG 400 was added to 100 ml pooled human urine and the mixture treated together with the samples as described above. The PEG-reference is henceforth referred to as PEG 400.

Gas-liquid chromatographic analysis of PEG

The columns were washed with a solution containing KOH (50 g/l in 90\% (w/w) ethanol), 500 ml warm water (about 60° C), 1000 ml glass-distilled water (about 22°C), and 1000 ml methanol. They were then dried at 150°C for 60 minutes and silanised with dimethyldichlorosilane in toluene (250 g/l). After packing, the column was put into the chromatograph and conditioned by heating to 380°C, maintaining this temperature for 10 minutes and cooling to 170°C. This procedure was repeated twice. The column was kept at 225°C for two days before use.
Instrument-quality nitrogen gas (N₂, AGA, Sweden) was used as carrier gas. The samples (1–10 μl) were injected at a column temperature of 170°C and isothermal conditions maintained for three minutes. Then, a temperature programme raised the temperature 6°C per minute up to 350°C. The detector temperature was 380°C. A single chromatograph run took about 30 minutes. Figure 1 shows a typical PEG-chromatogram consisting of 11 peaks. These correspond to polymers with molecular weights ranging from 194 daltons (four ethylene oxide units) to 634 daltons (14 ethylene oxide units). However, the 11th peak was not always detected. The area under each peak of interest was quantified by multiplying the width at half the peak height with the peak height.

**Precision in measurements**

The day-to-day coefficient of variation (CV) differed between the peaks. It was greatest, up to 29%, for the smaller peaks 1 and 10. The mean CV of six determinations on peaks 1–10 and on peaks 2–9 was 9% and 5.2%, respectively. The corresponding CVs for three different batches of PEG were 30.6% and 17.4%, respectively. The same batch of PEG should thus be used throughout an investigation.

**Mathematical operations**

The following operations were performed using the data from different PEG-chromatograms:

1. The relative peak area of the different sized molecular species were determined as described above. The percentage composition and median (m) molecular weight of the distribution were then calculated.

2. The distribution was nearly gaussian. After curve-fitting to the normal distribution, N(K) = (2πδ)⁻¹/₂ exp - ((K-m)²/2δ²), the mean (m) molecular weight of the distribution was estimated together with the standard deviation (SD). The least-squares method was used with the aid of a computer (Hewlett-Packard HP 9830).

3. By dividing the relative amount (=peak area) of each individual PEG recovered in the urine with the relative amount of that same PEG in the starting material, a relative barrier index for each molecular size was calculated. Also, the relative distribution of the indices was determined.

4. The distribution of the barrier indices was then adapted to a modification of Butterworth's filter functions²³, using an iterative, curve-fitting procedure with the least-squares method and a modified Marquardt's alogarithm²⁵, ²⁶. These operations were conducted with the aid of a computer (either Hewlett-Packard HP 9830 or Tectronix 4051 Graphic System).

The filter function has the principal formula

\[ F(K) = \text{constant} \cdot f_1(K) \cdot f_2(K) \]  

where K is the molecular weight, \(f_1(K)\) a filter function at lower molecular weights and \(f_2(K)\) a filter function at higher molecular weights.

The explicit formula of F is

\[ F(K) = H_0 \cdot \left( 1 - \frac{1}{H_1 + \left( \frac{K}{H_5} \right)^{H_6}} \right) \cdot \left( 1 - \frac{H_4}{1 + \left( \frac{K}{H_5} \right)^{H_6}} \right) \]  

where the different parameters have the following import:

- \(H_0\) = the amount that would be recovered in the urine if there were no filtering of molecules due to \(f_1(K)\) and \(f_2(K)\)
- \(H_1\) = the amount that passes through \(f_2(K)\)—that is, a measure of the strength of \(f_1(K)\)
- \(H_4\) = the breakpoint of \(f_2(K)\)—that is, the point at which \(f_2(K)\) has attained 50% of its maximum value
- \(H_5\) = the slope of \(f_2(K)\)—that is, the ranges of molecular sizes in which \(f_2(K)\) exerts its filtering effect
- \(H_6\) = the amount that passes through \(f_2(K)\)
- \(H_3\) = the breakpoint of \(f_1(K)\)

To get a condensed formula for the relative expression of \(f_2\), we made an estimator function, G, defined as

\[ G(K) = \frac{f_2,\text{max} - f_2(K)}{f_2,\text{max}} \cdot 100 \]  

The explicit formula of G is

\[ G(K) = \frac{H_4 \cdot 100}{1 + \left( \frac{H_5}{K} \right)^{H_6}} \]  

**Results**

**Characteristics of distribution of PEG**

A typical chromatogram for PEG 400 is shown in Fig. 1. Ten or (sometimes) 11 peaks were seen, corresponding to distinct polymeric units with molecular weights ranging from 194 to 634 daltons. The percentage composition of these different sized molecules is shown in Fig. 2, which also shows the best fit of the values to the normal distribution (total relative error for nine points less than 5%). This figure thus illustrates the approximate normal distribution of the different polymers.
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Table 1 summarises the characteristics of the PEG distribution before and after passage from intestine to urine. The urinary excretion in two different cases is shown. In the healthy state, the distribution was transposed towards lower molecular weights. By contrast, in the patient with Crohn's disease, the distribution was transposed towards higher molecular weights. The shift appeared in both the mean (m) and the median (m̃) values in both cases. However, this finding did not disclose whether the healthy individual excluded larger molecules alone or small molecules as well. Therefore, relative barrier indices for each polymer were calculated (Fig. 3). This procedure clearly demonstrated that, in the healthy state, two different filters appeared, one at lower molecular weights and one at higher. It also appeared that, in the patient with Crohn's disease, the filtering of smaller molecules was essentially the same as that in the healthy state but that there was no selective exclusion of larger molecules.

**Mathematical modelling of passage of PEG 400 from intestine to urine**

As two filtering functions appeared, the deterministic mathematical filter functions used to describe the passage from intestine to urine were made up from two functions, f₁ and f₄ (see Methods section). These were designed to determine the filtering efficiency at lower and higher molecular weights, respectively.

Table 2 summarises the values of $H₀$-$H₆$ for the healthy individual and the Crohn's disease patient.

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**Table 2** Parameters ($H₀$-$H₆$) of barrier function F in healthy state and Crohn's disease patient

<table>
<thead>
<tr>
<th>Subject</th>
<th>Parameters of F</th>
<th>$H₀$</th>
<th>$H₁$</th>
<th>$H₂$</th>
<th>$H₃$</th>
<th>$H₄$</th>
<th>$H₅$</th>
<th>$H₆$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy state</td>
<td></td>
<td>2.36</td>
<td>0.56</td>
<td>299</td>
<td>11.1</td>
<td>0.76</td>
<td>329</td>
<td>3.56</td>
</tr>
<tr>
<td>Crohn's disease patient</td>
<td></td>
<td>1.47</td>
<td>0.95</td>
<td>294</td>
<td>7.72</td>
<td>0.19</td>
<td>368</td>
<td>8.34</td>
</tr>
</tbody>
</table>

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**Fig. 1** A typical gas-liquid chromatogram for PEG 400.

**Fig. 2** Percentage composition of the polymers in PEG 400 and curve-fitting to the normal distribution.

**Fig. 3** Relative passage of the polymers in PEG 400 from the intestine to urine in a healthy state (■) and in a patient with Crohn's disease (○).
In the upper filter function $f_2$, the strength ($H_4$) was considerably smaller in the Crohn’s disease patient than in the healthy subject. Furthermore, the breakpoint occurred at a slightly higher value, 368 dalton, compared with 329 dalton, indicating that the transintestinal passage of larger molecules was greater in the diseased state. The relative expression of the upper barrier, as described by the G-function, was also significantly smaller in the Crohn’s disease patient than in the healthy individual (Table 3).

**Discussion**

The use of PEG 400 to characterise the intestinal permeability in man might offer a distinct advantage over other small molecules: the different-sized PEG molecules can serve as internal standard of the individual variations in each subject under study. Thus, systematic errors and irrelevant variables in each experiment are largely abolished. It should be stressed, again, that the purpose of the present investigation is to evaluate the possible use of this advantage rather than describing the human intestinal permeability in health and disease.

For PEG 400 absorption to be used to measure intestinal permeability, each individual subunit in the mixture should cross the intestinal wall at a rate inversely proportional to its molecular size. Furthermore, if the urinary excretion is to be used as a reliable measure of the intestinal permeability, the excretion of each molecular species should reflect the amount absorbed. We have found, however, that, after peroral administration of PEG 400 to healthy individuals as well as patients with gastrointestinal and joint diseases, low molecular weight PEG molecules are relatively less abundant in the urine than those of intermediate molecular weight (unpublished observation). This holds true also if PEG 400 is put into the cubital vein of healthy human beings or into the portal vein of rats (unpublished observation). All these findings provide evidence that all sizes of absorbed PEG molecules do not pass equally into the urine and that, although the first criterion mentioned above may be fulfilled, the second is not. We therefore suggest that PEG 400 should be used for determining intestinal permeability with the proviso that a more careful evaluation should be carried out than previously recommended.

We have invented a deterministic mathematical model to characterise both the intestinal and non-intestinal filters through which PEG must pass from the gut to the urine. A simplified compartment model to mimic the route is sketched in Fig. 4. After passage through the intestinal wall, the molecular profile in blood is changed through a loss of smaller PEG molecules to other compartments than the urine. A possible explanation of this loss is reabsorption of the smaller molecules by renal tubules.

![Simplified compartment model for the passage of PEG 400 from the intestine to urine.](image)

We therefore suggest that the overall passage from intestine to urine is characterised not only by the intestinal permeability barrier against the larger molecules (corresponding to $f_2(K)$) but also by the renal tubular reabsorption of the smaller molecules and/or their escape into other cells and tissues (corresponding to $f_1(K)$). Accordingly, a simple comparison of ratios of urinary-excreted, different-sized molecules cannot be used as reliable indices of intestinal permeability. Instead, at least two different functions ($f_2(K)$ and $f_1(K)$) should be considered and evaluated separately. This can be done, as shown in the present investigation, by using deterministic mathematical functions and computer-aided curve-fitting procedures. In this way, information is also obtained on alterations not attributable to gut permeability.

The present investigation illustrates the versatility of mathematical modelling in elucidating the various filters through which PEG must pass between the gut and the urine. As it was expected that one such filter would be the intestinal permeability barrier, we studied a patient with severe gastrointestinal disease (Crohn’s) rather than an apparently healthy individual. Our findings indicated that this patient had an increased intestinal permeability for the larger molecules. The break-point of the upper filter function was only slightly higher in the Crohn’s disease patient than in the healthy state. By contrast,
the barrier strength was considerably lower in this patient than in the healthy state. We therefore suggest that the increased intestinal permeability was due to a general impairment of the barrier function. The greater proportion of larger PEG molecules in the urine could theoretically reflect a change in the relative distribution of PEG absorption between jejunum, ileum, and colon, particularly as the patient had undergone two ileal and colonic resections. However, although each normal area of the intestine has different permeability characteristics, no area lacks filtering properties against PEG 400\textsuperscript{23}. It is therefore unlikely that a change in the relative distribution of different areas was responsible for not filtering larger PEGs. Rather it is likely that the patient’s intestinal barrier function was impaired. Unfortunately, our finding did not disclose whether the increased passage of larger PEGs occurred across ‘normal’ or ‘diseased’ intestine or both. This is a point of major interest, as, at the time of investigation, the inflammation was confined to terminal ileum, whereas the uptake of orally administered PEG 400 is thought to occur mainly in jejunum and proximal ileum\textsuperscript{23}. Our finding thus indicates that the intestinal barrier properties might be impaired also in areas that are not the site of severe inflammation or gross morphological alteration. It is possible that, during the course of Crohn’s disease, such areas are subsequently engaged. This hypothesis is now being further investigated by studying the relation between inflammatory activity and intestinal barrier properties in Crohn’s disease.

This research is supported by grants from the Swedish Medical Research Council (B78–16X–02183–12), from Tore Nilsson’s Fund for Medical Research, and from Carl-Bertel Nathorst’s Scientific and Social Foundations.

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doi: 10.1136/gut.21.3.208

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